



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>5</sup> :</b> <b>A61F 2/02, 2/28, 2/44</b> <b>A61K 9/14, 37/12, B32B 5/16</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 93/00050</b> <b>(43) International Publication Date:</b> 7 January 1993 (07.01.93)
<b>(21) International Application Number:</b> PCT/US92/05309 <b>(22) International Filing Date:</b> 22 June 1992 (22.06.92)  <b>(30) Priority data:</b> 718,721                      21 June 1991 (21.06.91)                      US  <b>(71) Applicant (for all designated States except US):</b> GENETICS INSTITUTE, INC. [US/US]; 87 Cambridge Park Drive, Cambridge, MA 02140 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only) :</b> RON, Eyal [US/US]; 71 Grant Street, Lexington, MA 02173 (US). TUREK, Thomas, J. [US/US]; 65 Bearse Avenue, Boston, MA 02124 (US). ISAACS, Benjamin, S. [US/US]; 75 William G. Drive, Tewksbury, MA 01876 (US). PATEL, Himakshi [US/US]; 21 Clever Lane, Tewksbury, MA 01876 (US). KENLEY, Richard, A. [US/US]; 10 Westminster Road, Andover, MA 01810 (US).		<b>(74) Agent:</b> McDANIELS, Patricia, A.; Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140 (US).  <b>(81) Designated States:</b> AU, BR, CA, FI, JP, KR, NO, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE).  <b>Published</b> <i>With international search report.          Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> PHARMACEUTICAL FORMULATIONS OF OSTEOGENIC PROTEINS  <b>(57) Abstract</b>  A composition comprising a pharmaceutically acceptable admixture of an osteogenic protein; a polymer matrix component selected from the group consisting of poly(lactic acid), poly(glycolic acid), and copolymers of lactic acid and glycolic acid; and an osteogenic protein-sequestering material.		

BEST AVAILABLE COPY

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	MI	Mali
AU	Australia	FR	France	MN	Mongolia
BB	Barbados	GA	Gabon	MR	Mauritania
BE	Belgium	GB	United Kingdom	MW	Malawi
BF	Burkina Faso	GN	Guinea	NI	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IE	Ireland	RO	Romania
CA	Canada	IT	Italy	RU	Russian Federation
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	SN	Senegal
CI	Côte d'Ivoire	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TC	Togo
DE	Germany	MC	Monaco	US	United States of America
DK	Denmark	MG	Madagascar		
ES	Spain				

5

TITLE OF THE INVENTION

## PHARMACEUTICAL FORMULATIONS OF OSTEOGENIC PROTEINS

BACKGROUND OF THE INVENTION

10       The subject invention relates to the field of osteogenic  
proteins and pharmaceutical formulations thereof. More  
particularly, the subject invention involves pharmaceutical  
formulations designed to sequester osteogenic protein in-situ for  
a time sufficient to allow the protein to induce cartilage and/or  
15 bone formation.

Osteogenic proteins are those proteins capable of inducing, or  
assisting in the induction of cartilage and/or bone formation.  
Many such osteogenic proteins have in recent years been isolated  
and characterized, and some have been produced by recombinant  
20 methods. For example, so-called bone morphogenic proteins (BMP)  
have been isolated from demineralized bone tissue (see e.g. Urist  
U.S. 4,455,256); a number of such BMP proteins have been produced  
by recombinant techniques (see e.g. Wang et al. U.S. 4,877,864 and  
Wang et al. U.S. 5,013,549); a family of transforming growth  
25 factors (TGF- $\alpha$  and TGF- $\beta$ ) has been identified as potentially useful  
in the treatment of bone disease (see e.g. Derynck et al., EP  
154,434); a protein designated Vgr-1 has been found to be expressed  
at high levels in osteogenic cells (see Lyons et al. (1989) Proc.  
Nat'l. Acad. Sci. USA 86, 4554-4558); and proteins designated OP-1,  
30 COP-5 and COP-7 have purportedly shown bone inductive activity (see  
Oppermann, et al. U.S. 5,001,691).

Various attempts have been made at developing formulations  
designed to deliver osteogenic proteins to a site where induction  
of bone formation is desired. For example, certain polymeric  
35 matrices such as acrylic ester polymer (Urist, U.S. 4,526,909) and  
lactic acid polymer (Urist, U.S. 4,563,489) have been utilized, but  
these formulations do not sequester the osteogenic protein for a

time sufficient to optimally induce bone formation and further have been found to erode too slowly for optimal bone formation.

A biodegradable matrix of porous particles for delivery of an osteogenic protein designated as OP is disclosed in Kuberasampath, U.S. 5,108,753. While US 5,108,753 discloses that a successful carrier for OP must bind the protein, act as a slow release delivery system, accommodate each step of the cellular response during bone development, and protect the protein from nonspecific proteolysis, no formulations are suggested which contain components that specifically sequester the OP at the site where bone formation is desired.

Okada et al., US 4,652,441, US 4,711,782, US 4,917,893 and US 5,061,492 and Yamamoto et al., US 4,954,298 disclose a prolonged-release microcapsule comprising a polypeptide drug and a drug-retaining substance encapsulated in an inner aqueous layer surrounded by a polymer wall substance in an outer oil layer. Although bone morphogenic protein is listed as a polypeptide capable of such a formation, microencapsulation of osteogenic proteins prevents controlled release of such protein sufficient for optimal bone formation.

Collagen matrices have also been used as delivery vehicles for osteogenic proteins (see e.g. Jeffries, U.S. 4,394,370), but collagen frequently causes undesirable antigenic reactions in patients. Therefore, there remains a need for a pharmaceutical formulation capable of sequestering osteogenic proteins at a site where induction of bone formation is desired for a time sufficient to allow safe, effective induction of such bone formation.

#### SUMMARY OF THE INVENTION

In one embodiment, the subject invention provides a composition comprising a pharmaceutically acceptable admixture of an osteogenic protein; a polymer matrix component selected from the group consisting of poly(lactic acid), poly(glycolic acid), and copolymers of lactic acid and glycolic acid; and an osteogenic protein-sequestering alkylcellulose.

In another embodiment, the subject invention provides a composition comprising a pharmaceutically acceptable admixture of an osteogenic protein; a polymer matrix component selected from the group consisting of poly(lactic acid), poly(glycolic acid), and copolymers of lactic acid and glycolic acid; and an osteogenic protein-sequestering agent selected from the group consisting of hyaluronic acid, alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer, and poly(vinyl alcohol).

In another embodiment, the subject invention provides a composition comprising polymeric particles having a spherical diameter of between about 150 and 850 microns and a porosity such that the surface area of the particles is between about 0.02 and 4 m<sup>2</sup>/g, wherein the polymer is selected from the group consisting of poly(lactic acid), poly(glycolic acid), and copolymers of lactic acid and glycolic acid; optionally in admixture with osteogenic protein.

In yet another embodiment, the subject invention provides a composition comprising a pharmaceutically acceptable admixture of osteogenic protein and an effective solubilizing amount of a member selected from the group consisting of arginine, histidine, dextran sulfate, gamma-amino butyric acid, beta-amino propionic acid, glycine-glycine, glycine ethyl ester, histidine ethyl ester, lysine methyl ester, arginine methyl ester, guanidine, sodium chloride, heparin, lysine, beta-alanine ethyl ester and agmatine.

#### DETAILED DESCRIPTION OF THE INVENTION

The osteogenic proteins useful in the practice of the subject invention are well known to those skilled in the art and include those discussed above. The preferred osteogenic proteins for use herein are those of the BMP class identified as BMP-1 through BMP-8 in U.S. 4,877,864; U.S. 5,013,649; WO 90/11366 published October 4, 1990; and WO 91/18098 published November 28, 1991. The most preferred is BMP-2, the mature protein sequence beginning with the

amino acid Gln at nucleotide 1202 and ending with the amino acid Arg at nucleotide 1543, as described in detail in the '649 patent. Of course, combinations of two or more of such osteogenic proteins may be used, as may fragments of such proteins that also exhibit osteogenic activity and heterodimeric forms of such proteins. Recombinant proteins are preferred over naturally occurring isolated proteins. The amount of osteogenic protein useful herein is that amount effective to stimulate increased osteogenic activity of infiltrating progenitor cells, and will depend upon the size and nature of the defect being treated as discussed in more detail below, such amounts being orders of magnitude less than the amount of polymer matrix employed, preferably in the range of 1-50  $\mu$ g of protein for each 10 mg of polymer matrix employed and more preferably in the range of 0.5 - 5  $\mu$ g protein for each mg of polymer matrix employed.

The osteogenic proteins can be utilized in the form of a pharmaceutically acceptable solution or in lyophilized form. In either case it is optimal to stabilize and solubilize the osteogenic protein, preferably at concentrations of at least 1 mg/ml so that a pharmaceutically effective amount of protein can be delivered without undue volumes of carrier solution being necessary. However, a problem exists in that osteogenic proteins, particularly those of the BMP family, have proven to be difficult to solubilize. As detailed in the examples below, it has been discovered that amino acids having a net positive charge (e.g. net 1+ species such as arginine, histidine, lysine and the ethyl esters of glycine and beta-alanine), preferably a net 2+ charge (e.g. the ethyl ester of histidine, the methyl esters of lysine and arginine, and agmatine), are useful in this regard. Amino acids having a net zero charge are useful in this regard provided that the positive charge of the compound is sufficiently distant (at least 2-3  $\text{CH}_2$  units away) from the neutralizing negative charge (e.g. net neutral species such as gamma-amino butyric acid, beta-amino propionic acid and glycine-glycine dipeptide). Other solubilizing agents useful

herein include dextran sulfate, guanidine, heparin and sodium chloride. For use in solubilizing BMP-2, the preferred solubilizing agents are arginine and histidine (including esters thereof). Arginine is used in concentrations of about 50-600 mM, preferably 300-500 mM. Histidine may be added to arginine to solubilize BMP-2, in concentrations of about 1-100 mM, preferably 10-50 mM. When histidine is used alone as a solubilizing agent, it is used in concentrations of about 50-600 mM, preferably 300-500 mM. Various well known methods may be used to compound the osteogenic protein and solubilizing agents for use herein, including but not limited to ultrafiltration, dialysis, gel filtration, and hydrophobic interaction chromatography.

The polymer matrix component useful in the practice of the subject invention is a polymeric material that can be formed into porous particles as described below thereby providing in-situ scaffolding for the osteogenic protein, while having biodegradable properties allowing for replacement by new bone growth. Examples are polymers of amino acids, orthoesters, anhydrides, propylene-co-fumarates, or a polymer of one or more  $\alpha$ -hydroxy carboxylic acid monomers, e.g.  $\alpha$ -hydroxy acetic acid (glycolic acid) and/or  $\alpha$ -hydroxy propionic acid (lactic acid). The latter can be employed in its d- or l- form, or as a racemic mixture, the racemic mixture being preferred. When a copolymer of lactic acid and glycolic acid is employed (PLGA), the molar ratio of monomers can range from 1:99 to 99:1 depending upon the desired bioerosion lifetime which in turn depends upon the clinical indication being addressed, as more than 50% of either monomer gives longer bioerosion lifetime (slower biodegradation). The molecular weight of the polymer can range from about 1,000 to 100,000 with 30,000-50,000 being preferred when a 50:50 copolymer is employed. The higher the molecular weight the slower the biodegradation.

The polymeric matrix component of the subject invention is used in the form of highly porous to hollow (with surface porosity) particles, hereinafter collectively referred to as "porous particles." These porous particles are generally spherical having

5 diameters of 150 to 850 microns. This particle size creates sufficient spacing between particles to allow mammalian osteoprogenitor cells to infiltrate and be positively influenced by the osteogenic protein (evidenced by an increase in osteogenic activity/bone growth rate).

10 While it has generally been postulated that particles suitable as matrices for delivery of osteogenic proteins should be porous, the extent of porosity necessary to optimally induce bone formation has not previously been studied. The present inventors have discovered that the average surface area per porous particle is critical to optimize bone formation. Specifically, porous particles useful in bone formation according to the present invention should have an average surface area of from about 0.02 to 4 m<sup>2</sup>/g. The present inventors have further discovered that it is possible to produce porous particles having the desired surface area by introducing a "porosigen" (composition capable of imparting porosity by increasing particle surface area) into the solution used to produce the porous particles. It is also possible to control the bioerosion rate by subjecting the porous particles to sterilizing doses of  $\gamma$  radiation. The higher the  $\gamma$  radiation dose, the faster the bioerosion.

25 The method of producing porous particles in accordance with the subject invention and discussed hereinbelow results in particles having a porosity such that the surface area of the particles is increased about 2-250 fold over the surface area of non-porous particles of comparable size. More specifically, e.g., non-porous PLGA particles having an average size of 400 $\mu$ m have a surface area of 0.018 m<sup>2</sup>/g. In contrast, PLGA particles useful in the subject invention made utilizing 50% NaCl as a porosigen have a surface area of between about 0.2 and 0.6 m<sup>2</sup>/g; and particles made using sucrose as a porosigen have a surface area of between about 0.04 and 0.09 m<sup>2</sup>/g as described in Example 1. PLGA particles of the present invention made using liquid porosigen with homogenization as described in Example 2 have a surface area of between about 0.02



and 4 m<sup>2</sup>/g.

A preferred method of production of the porous particles of the invention is, generally speaking, a solvent evaporation process comprising dissolving the polymer (in e.g. CH<sub>2</sub>Cl<sub>2</sub>), and adding a  
5 porosigen such as NaCl, mannitol or sucrose in solid and/or liquid form. When porosigen is added in solid form, the matrix-porosigen solution takes the form of a suspension. Another preferred method of production of the porous particles of the invention is a solvent extraction method, wherein the porosigen is added in liquid form  
10 with concomitant homogenization. When porosigen is added in liquid form with homogenization, the matrix-porosigen solution takes the form of an emulsion. With either method, the matrix-porosigen emulsion is added to an excess aqueous solution containing surfactant such as poly(vinyl alcohol) with controlled stirring and  
15 temperature. The resultant porous particles are hardened by extracting or evaporating residual solvent, and dried.

The porous nature of the particles of the present invention creates sufficient surface area for protein adsorption and increases biodegradation, the desirable extent of both being  
20 dependent upon the clinical indication being addressed. Surface area can be measured by any conventional technique. For example, BET surface area analysis can be employed using a Micromeritics ASAP 2000 system as is explained in more detail in Examples 1 and 2 below. The amount of porous particles used to treat a particular  
25 defect will, of course, depend upon the size of the defect being treated, and on the effective amount required to adsorb the osteogenic protein.

The osteogenic protein-sequestering material useful in the practice of the subject invention is a pharmaceutically acceptable  
30 material having a viscosity and polarity such that, when added to an osteogenic protein/porous particle combination, a malleable (putty-like) composite is formed that handles appropriately for surgical implantation into an injury site. Adding the sequestering agent to the combination of bioerodible porous particles plus

osteogenic protein contains the adsorbed protein within the matrix for a time sufficient to allow the protein to increase the otherwise natural rate of osteogenic activity of the infiltrating mammalian progenitor cells. The sequestering material further  
5 allows the osteogenic protein to diffuse from the malleable composite over a time interval appropriate for optimally increasing the rate of osteogenic activity of the progenitor cells. In the absence of such a sequestering material, osteogenic protein desorbs from the PLGA particles in-situ at a rate such that the  
10 osteoinducing effect of the protein is not clinically significant.

A preferred family of sequestering agents is cellulosic materials such as alkylcellulose (including hydroxyalkylcellulose), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, and  
15 carboxymethylcellulose, the most preferred being the cationic salts of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20  
20 wt%, preferably 1-10 wt% based on total formulation weight, which represents the amount necessary to prevent desorption of the osteogenic protein from the polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix,  
25 thereby providing the protein the opportunity to assist the osteogenic activity of the progenitor cells.

Additional optional components useful in the practice of the subject application include, e.g. cryogenic protectors such as mannitol, sucrose, lactose, glucose, or glycine (to protect the  
30 osteogenic protein from degradation during lyophilization), antimicrobial preservatives such as methyl and propyl parabens and benzyl alcohol; antioxidants such as EDTA, citrate and BHT (butylated hydroxytoluene); and surfactants such as poly(sorbates) and poly(oxyethylenes); etc.

35 According to the present invention, the osteogenic protein is

not included in the PLGA polymerization solution or encapsulated in PLGA microcapsules but is added to the already polymerized porous particles. It is preferable to add the porous particles to the solution of osteogenic protein prior to addition of sequestering agent in order to allow the protein to adsorb onto the particles. Of course, the traditional preparation of formulations in pharmaceutically acceptable form (i.e. pyrogen free, appropriate pH and isotonicity, sterility, etc.) is well within the skill in the art and is applicable to the formulations of the subject invention. The formulations may be provided to the clinic as a single vial formulation, either as a solution or in lyophilized form, or the formulation may be provided as a multicomponent kit wherein, e.g. the osteogenic protein is provided in one vial and the porous particles and sequestering agent are provided in a separate vial or vials.

As seen in Examples 4 and 5 below, the formulations of the subject invention provide malleable implants that allow therapeutically effective amounts of osteoinductive protein to be delivered to an injury site where cartilage and/or bone formation is desired. Such an implant may be used as a substitute for autologous bone graft in fresh and non-union fractures, spinal fusions, and bone defect repair in the orthopaedic field; in cranio/maxillofacial reconstructions; for prosthesis integration, especially as a surface coating; in osteomyelitis for bone regeneration, and in the dental field for erosion of the alveolar ridge and periodontal disease. In certain of these uses, the compositions of the subject invention may be used in combination with various bone cements, including erodible bone cements such as poly(propylene-co-fumarate). The lower viscosity formulations may also be used as a percutaneous injection to accelerate healing of closed fractures. As alluded to above, the dosage regimen will be determined by the clinical indication being addressed, as well as by various patient variables (e.g. weight, age, sex) and clinical presentation (e.g. extent of injury, site of injury, etc.).

Currently, fresh autogeneic bone is widely used as a bone

graft material. The limited supply of autogeneic bone, along with the necessity for an additional harvesting surgical procedure, constitute major disadvantages in use of autogeneic bone for bone grafting. In accordance with the present invention, the porous particles of the invention may be added to autogeneic bone to extend the amount of material available for bone grafting. The porous particles may also be used in combination with a sequestering agent as a substitute for bone wax at the site of a bony injury to act as a bioerodible hemostat.

#### EXAMPLES

All components utilized in these examples are pharmaceutical grade. The polymeric particle component was made from a 50:50 (molar) random copolymer of lactic acid and glycolic acid (PLGA) having a weight average molecular weight of 30,000-50,000, a number average molecular weight of about 20,000 (by gel permeation chromatography relative to polystyrene standards), and an inherent viscosity of 0.35-0.45 dL/g. The osteogenic protein utilized was rBMP-2. The production and characterization of rBMP-2 is described in detail in the above-referenced US 5,013,649. The sequestering agents utilized included carboxymethyl-cellulose, hydroxy-propylmethyl cellulose, sodium alginate, hyaluronic acid, and poly(ethylene glycol). The carboxymethylcellulose (CMC) utilized contained 0.7 degree of substitution (carboxymethyl groups per hydroxy group on cellulose and had a viscosity of 2480 cps.

#### EXAMPLE 1 - PREPARATION OF POROUS PARTICLES BY SOLVENT EVAPORATION TECHNIQUE

PLGA was dissolved in  $\text{CH}_2\text{Cl}_2$  (15% w/v), and 10 g porosigen (7.5% w/v) was suspended in this solution. The resulting solution was added to an excess poly(vinyl alcohol) aqueous solution (0.1% w/v). After a few hours of stirring under partial vacuum (24 inches Hg), the particles were hardened in excess cold ethanol (95%). The resulting particles were washed with water for

injection and vacuum dried to give a free-flowing product. BET surface area analysis was performed using a Micrometrics ASAP 2000 system. The measurement of surface area is based upon adsorption and desorption of Krypton gas at the surface and within the pores of the solid sample. The unit calculates and prints out the surface area:

$$\frac{1}{VA[(P_0/P)-1]} = \frac{C-1}{V_m C} (P/P_0) + \frac{1}{V_m C}$$

- 10 V = volume absorb at pressure P       $P_0$  = saturation pressure  
 P/P<sub>0</sub> = relative pressure      P = pressure  
 C = constant      A = gas cross sectional area  
 V<sub>m</sub> = Monolayer Capacity

15 By plotting  $\frac{1}{VA[(P_0/P)-1]}$  vs P/P<sub>0</sub>, the slope being

$\frac{C-1}{V_m C}$  and the intercept being  $\frac{1}{V_m C}$ , the surface area

- 20  $S_i = \frac{V_m N_A}{V}$  where N = Avogadro's number and V = molar volume.

Reactant details and results are depicted in Table 1 and Table 2, respectively.

25

Table 1

	<u>Batch No.</u>	<u>PLGA (grams)</u>	<u>CH<sub>2</sub>CL<sub>2</sub> (mL)</u>	<u>Porosigen (type/%)</u>	<u>PVA (mL)</u>	<u>Impellers (top/btm)</u>	<u>Stirring (rpm)</u>
5	1	10	67	NaCl/50	1200	(2rshtn/A-100)	215
	2	10	67	NaCl/80	1200	(2rshtn/A-100)	215
10	3	6.7	67	suc/50	1200	(2rshtn/A-100)	215
	4	6.7	67	NaCl/50	1200	(2rshtn/A-100)	235
	5	16	106	suc/50	2000	(A-310/A-310)	140
15	6	20	133	suc/50	2000	(A-310/A310)	140
	7	20	133	suc/50	2000	(A-310/A-310)	140
20	8	20	133	suc/50	2000	(8.5rsh/A-310)	100
	9	20	133	suc/50	2000	(8.5rsh/A-310)	140
	10	20	133	suc/50	2000	(2rshtn/A-100)	140
25							

Table 2

	<u>Batch No.</u>	<u>Mn</u>	<u>Mw</u>	<u>Dp</u>	<u>Surface Area (m<sup>2</sup>/g)</u>	<u>Yield % 250-710μm</u>	<u>Density (gms/cc)</u>
5	1	17500	30800	1.75	0.54	27.3	0.41
	2	19400	31700	1.64	0.037	71.6	0.67
10	3	19700	40900	2.07	0.089	92.7	0.70
	4	20300	37700	1.86	0.28	69.5	0.37
	5	20400	32600	1.60	0.035	N/A	N/A
15	6	20200	37700	1.86	0.079	79.5	0.64
	7				0.060	85	0.76
20	8				0.038	85	0.86
	9				0.057	65	0.71
25	10	20200	37700	1.86	0.060	64	0.68

EXAMPLE 2 - PREPARATION OF POROUS PARTICLES  
BY SOLVENT EXTRACTION TECHNIQUE

A 100-g sample of PLGA was dissolved in 670 mL of  $\text{CH}_2\text{Cl}_2$ .  
5 The porosigen solution was prepared by dissolving 5g NaCl in 50 mL of a 0.2% aqueous solution of poly(vinyl alcohol). A 50-mL aliquot of the porosigen solution was added to a homogenizer and agitated at 3300 rpm. The PLGA solution was then added to the homogenizer (with agitation). A 77 mM NaCl solution in 10 liter  
10 of 0.2% aqueous poly(vinyl alcohol) was added to a 12-liter reactor and agitated at 175 rpm. The PLGA/porosigen suspension was added to the reactor over 90 minutes. A 77 mM NaCl solution in 0.2% aqueous poly(vinyl alcohol) was then pumped into and out of the 12-liter reactor, thereby extracting methylene chloride  
15 from the mixture. After solvent extraction was complete, agitation was stopped, the porous particles are allowed to settle, the supernatant was decanted, and the particles were hardened with ethanol (95%) followed by washing with water or a solution of polysorbate 20 (0.05%) in water. The washed  
20 particles were dried by vacuum or convection techniques. Particles prepared according to this procedure typically have 0.09 g/cc bulk density, and total surface area =  $4 \text{ m}^2/\text{g}$ . The dried particles can be sterilized by ethylene oxide exposure or  $\gamma$  irradiation. As noted above, the  $\gamma$  radiation dose influences  
25 bioerosion rate. Table 3 gives examples of porous particles prepared by the above process.



<u>Table 3</u>					
	<u>Batch No.</u>	<u>Homogenizer (rpm)</u>	<u>Porosigen Soln, (mL)</u>	<u>Density (g/cc)</u>	<u>Yield % 150-500 <math>\mu</math>m</u>
5	1	10,000	50	0.09	80
	2	3,500	50	0.15	80
10	3	6,000	50	0.09	95
	4	3,000	50	0.10	95
15	5	1,900	50	0.29	90
	6	2,200	50	0.10	95
	7	2,000	50	0.09	99
20	8	2,000	25	0.14	90
	9	2,000	12.5	0.24	90
25	10	2,000	20	0.28	<75
	11	2,000	20	0.18	>75
	12	2,000	17.5	0.16	95
30	13	2,000	15.5	0.21	94
	14	2,500	15.5	0.17	90
35	15	2,200	15.5	0.19	90

EXAMPLE 3 - SOLUBILIZATION OF PROTEIN

Solubility of rBMP-2 in the excipients listed in Table 4 below was determined by dialysis in accordance with the following. Concentrations were determined by absorbencies at 280 nm using an extinction coefficient of 1.62. Protein solution (1 ml) containing 2-3 mg/ml of protein, 0.5M arginine and 10mM phosphate (pH 6.5) was dialyzed against 1000 ml of buffer containing 0.5M excipient of choice (see Table 3) and 0.5M arginine, pH 6.5. The dialysis was carried out at room temperature. The excipients were allowed to equilibrate with the protein solution. The protein solution was then dialyzed twice against 1000 ml of an arginine-less buffer solution of otherwise identical composition. Solubility results are presented in Table 4. Unless otherwise indicated, excipients were tested at standard concentrations of 500mM.

<u>Table 4</u>		
<u>EXCIPIENT</u>	<u>NET CHARGE</u> <u>(at neutral pH)</u>	<u>SOLUBILITY</u> <u>(mg/ml)</u>
ε-amino caproic acid	0	<0.4
5 δ-amino valeric acid	0	<0.4
γ-amino butyric acid	0	≥1.7
β-amino propionic acid	0	≥1.1
Glycine-Glycine dipeptide	0	≥1.8
Glycine	0	≤0.4
10 Arginine	1+	≥5.4
Lysine	1+	≥0.9
Guanidine	1+	≥1.8
Glycine (ethyl ester)	1+	≥2.2
Histidine (ethyl ester)	2+	≥2.2
15 Lysine (methyl ester)	2+	≥2.2
Arginine (methyl ester)	2+	≥2.2
Histidine	1+	≥2.2
Dextran sulfate		≥1.7
1.0M NaCl	0	≥1.8

EXAMPLE 4 - IMPLANT ANALYSIS

5 rBMP-2 (22  $\mu$ g), mannitol (8 mg), and epsilon aminocaproic acid (2M, 20 $\mu$ l) were lyophilized onto the PLGA particles (10 mg, 20% porosity, 325 mm). CMC (5.5 mg, ~9%) was added and the solid powder was sterilized using ethylene oxide. Water for Injection (60  $\mu$ l) was added to form a malleable implant of the composite. As a control, the same formulation was made without the CMC, in which case a gelatin capsule was used to hold the formulation in place. Both formulations were implanted in a 5 mm rat femur defect. The rats were sacrificed after twelve weeks. Ex-vivo analysis of the new bone was performed radiographically relative to the contralateral femur. Surprisingly, 83% (10 of 12) of the femur defects showed union utilizing the formulations of the subject invention, compared with only 50% (4 of 8) for the control.

EXAMPLE 5 - IMPLANT ANALYSIS

20 A 300- $\mu$ L aliquot of 0.12 mg/mL rBMP2 solution (in 0.25 M arginine, 10 mM histidine, pH 6.5, plus 20 mM calcium chloride) was added to 9.6 mg of porous particles (0.16 g/cc density, surface area = approximately 0.8 m<sup>2</sup>/g, sterilized by 2.5 Mrad  $\gamma$  radiation). To this mixture was added 15 mg sodium alginate. Gentle mixing provided a malleable composite. Similar formulations were made using 9 mg of hydroxypropylmethylcellulose or 9 mg carboxymethylcellulose except that the 0.12 mg/mL rBMP2 was solubilized in 0.25 M arginine plus 10 mM histidine, pH 6.5 (no added calcium chloride). As controls, malleable formulations

were prepared with 0.25 M arginine plus 10mM histidine containing 0 mg/mL rBMP2. The formulations were implanted into 8 mm diameter critical size circular defects in rat calvarium. After 21 days the animals were sacrificed and bone regeneration assessed by radiomorphometry (X-OMATL high contrast X-ray film, using Cambridge 520 Image Analysis System). The control samples for alginate, CMC, and hydroxypropylmethyl-cellulose formulations, respectively, showed only 18%, 10% and 10%, radiopacity. By comparison the alginate, CMC and hydroxypropylmethylcellulose formulations (with added rBMP2) showed, respectively, 72%, 70% and 67% radiopacity indicating significant new bone growth.

What is claimed is:

1. A composition comprising a pharmaceutically acceptable admixture of
  - (i) an osteogenic protein;
  - (ii) a polymer matrix component selected from the group consisting of poly(lactic acid), poly(glycolic acid), and copolymers of lactic acid and glycolic acid; and
  - (iii) an osteogenic protein-sequestering alkylcellulose.
2. The composition of claim 1 wherein the osteogenic protein is selected from the group consisting of the members of the BMP-family.
3. The composition of claim 2 wherein the osteogenic protein is BMP-2.
4. The composition of claim 2 wherein the cellulosic material is selected from hydroxypropylmethylcellulose and carboxymethylcellulose.
5. The composition of claim 3 wherein the cellulosic material is selected from hydroxypropylmethylcellulose and carboxymethylcellulose.
6. The composition of claim 5, wherein the polymer matrix component is a copolymer of lactic acid and glycolic acid.

7. The composition of claim 1 wherein the polymer matrix component is in the form of porous particles.
8. The composition of claim 2 wherein the polymer matrix component is in the form of porous particles.
9. The composition of claim 3 wherein the polymer matrix component is in the form of porous particles.
10. The composition of claim 4 wherein the polymer matrix component is in the form of porous particles.
11. The composition of claim 5 wherein the polymer matrix component is in the form of porous particles.
12. The composition of claim 6 wherein the polymer matrix component is in the form of porous particles.
13. A composition comprising a pharmaceutically acceptable admixture of
  - (i) BMP-2;
  - (ii) a polymeric matrix component comprising polymeric particles having a diameter of between about 150 and 850 microns and a porosity such that the surface area of the particles is between about 0.02 and 4 m<sup>2</sup>/g,

wherein the polymer is selected from the group consisting of poly(lactic acid), poly(glycolic acid), and copolymers of lactic acid and glycolic acid; and (iii) a protein sequestering amount of carboxymethylcellulose.

14. The composition of claim 1 wherein the osteogenic protein is TGF- $\beta$ .

15. The composition of claim 1 wherein the osteogenic protein is Vgr-1.

16. The composition of claim 1 wherein the osteogenic protein is OP-1.

17. The composition of claim 1 wherein the osteogenic protein is selected from COP-5 and COP-7.

18. A composition comprising polymeric particles having a spherical diameter of between about 150 and 850 microns and a porosity such that the surface area of the particles is between about 0.02 and 4 m<sup>2</sup>/g, wherein the polymer is selected from the group consisting of poly(lactic acid), poly(glycolic acid), and copolymers of lactic acid and glycolic acid.

19. A composition comprising polymeric particles as defined in



claim 18 in admixture with osteogenic protein.

20. A composition comprising a pharmaceutically acceptable admixture of osteogenic protein and an effective solubilizing amount of a member selected from the group consisting of arginine, histidine, dextran sulfate, gamma-amino butyric acid, beta-amino propionic acid, glycine-glycine, glycine ethyl ester, histidine ethyl ester, lysine methyl ester, arginine methyl ester, guanidine, sodium chloride, heparin, lysine, beta-alanine ethyl ester and agmatine.

21. A composition comprising a pharmaceutically acceptable admixture of

- (i) an osteogenic protein;
- (ii) a polymer matrix component selected from the group consisting of poly(lactic acid), poly(glycolic acid), and copolymers of lactic acid and glycolic acid; and
- (iii) an osteogenic protein-sequestering agent selected from the group consisting of hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer, and poly(vinyl alcohol).

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US92/05309**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/ 422, 423, 426, 484, 486, 489, 490, 497, 498; 514/2, 21, 773; 428/402.2, 402.21; 530/353, 840; 623/16, 17

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,917,893 (OKADA et al) 17 April 1990, col. 1-2; col. 2, lines 64-65; col. 5, lines 5 and 22-24	1-20
A	US, A, 4,637,931 (SCHMITZ) 20 January 1987, col. 3, lines 47-52	1-20

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

•	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"Z"	document member of the same patent family

Date of the actual completion of the international search 06 August 1992	Date of mailing of the international search report <b>03 NOV 1992</b>
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer <i>Carlos Azpuru</i> CARLOS AZPURU Telephone No. (703) 308-0237

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/05309

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (5):

A 61 F 2/02, 2/28, 2/44; A 61 K 9/14, 37/12; B 32 B 5/16

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/ 422, 423, 426, 484, 486, 489, 490, 497, 498; 514/2, 21, 773; 428/402.2, 402.21; 530/353, 840; 623/16, 17

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**